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Molecular diagnosis of potentially human pathogenic *Enterocytozoon bieneusi* and *Encephalitozoon* species in exotic birds in Southwestern Iran

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ABSTRACT

Microsporidia are obligate intracellular parasites that produce spores. The infections caused by these parasites are mostly considered to be opportunistic in immunodeficient patients. Because of the zoonotic nature of microsporidia as well as the increasing prevalence of immunodeficiency diseases, the aim of this study was to evaluate the molecular diagnosis of *Enterocytozoon bieneusi* (*E. bieneusi*) and *Encephalitozoon* spp. in exotic birds in southwestern Iran. Initially, 816 stool specimens were collected and stained by modified trichrome (Weber) staining. The slides were explored using light microscopy. In the next stage, the extracted DNA was amplified using a multiplex/nested PCR method. RFLP with the MnlI restriction enzyme was used to differentiate the *Encephalitozoon* species in the products of the molecular analysis. Out of 816 samples, 138 and 181 cases were found to be positive by the staining and the multiplex/nested-PCR methods, respectively. Of the 181 samples, 103 and 78 samples were positive for *E. bieneusi* and *Encephalitozoon* spp., respectively. The *Encephalitozoon* species were 17 *E. cuniculi*, 52 *E. intestinalis* and 9 *E. hellem*. Of 103 *E. bieneusi* samples, 57, 39, 2 and 5 cases were detected as genotypes D, M, E and L, respectively. The results showed a relatively high prevalence of microsporidia in exotic birds, and according to the results of the genotyping, these birds can be an important source of microsporidiosis. It is essential that high-risk individuals, including patients with immunodeficiency diseases, receive accurate and valid information about the risk of direct and indirect contact with infected exotic birds.

Introduction

Microsporidia are obligate intracellular parasites. Although the phylum microsporidia consists of 150 genera and 1200 species, *Encephalitozoon* spp. (including *E. intestinalis, E. hellem,* and *E. cuniculi*) and *Enterocytozoon bieneusi* (*E. bieneusi*) are the most frequent causes of human microsporidiosis [1]. These microorganisms can cause infection in both a wide range of animals and humans [2,3]. The life cycle of the parasite consists of 3 stages: the infectious stage, the growing or replicating stage (schizogony) and the spore formation stage (sporogony) [4]. The symptoms of microsporidiosis include myositis, keratoconjunctivitis, hepatitis, sinusitis, disseminated infections [5], chronic diarrhea, severe weight loss, nausea and confusion [6]. It is possible that these parasites are transferred through water contaminated with animal stool [7].

In recent years, *Encephalitozoon* spp. and *E. bieneusi* have been detected in birds. Birds are considered as the primary hosts of *E. hellem* [8]. For example, the parasite was found in ducks, pigeons, geese, crows, puffins, hummingbirds, swans and cranes [1,8–11] and in captive birds from the order Psittaciformes, which includes lovebirds, budgerigars, Eclectus parrots, parrots, cockatoos and lories. *E. hellem* has also been identified in ostriches and Gouldian finches [1,12–16]. In addition, *E. cuniculi* has been detected in cockatiels, chickens, and pigeons [10,17,18], and *E. intestinalis* has been found in pigeons and geese [9–11]. Furthermore, *E. bieneusi* has been identified in chickens, grey parrots, pigeons, cockatiels, lovebirds, finches, falcons and other birds [10,19–23]. These examples

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Table 1
The exotic bird families and their species included in the current study.

Exotic bird families	Species
Fringillidae	*Carduelis spinus; Carduelis flavirostris; Linchura cantans*
Canary	*Serinus canarius canarius*
Psittacidae	*Psittacula eupatria or Psittacus erithacus*
African grey parrot	*Psittacula krameri*
Budgerigar	*Melopsittacus undulatus*
White-eared Bulbul	*Pycnonotus leucotis*
Passeriformes or Myna	*Acridotheres tristis*

imply the zoonotic potential of microsporidia [24]. Notably, exposure to pigeons may be the significant link in the epidemiology of human microsporidiosis [9], as well as exposure to pet birds of some patients with ocular microsporidiosis [25]. On the other hand, these parasites can be life-threatening in immunodeficient individuals [2,7]. Hence, because of the zoonotic nature of microsporidia as well as the increasing prevalence of immunodeficiency diseases, the aim of this study was to evaluate the molecular diagnosis of *E. bieneusi* and *Encephalitozoon* spp. in exotic birds in southwestern Iran.

Methods

Sample collection

Initially, 816 fecal specimens were collected from several pet shops and houses in Ahvaz city, Khuzestan province, southwestern Iran, during the period 2012–2014. Table 1 shows the exotic bird families and their species included in the current study. The collected samples were transferred to the Department of Parasitology, Ahvaz Jundishapur University of Medical Sciences. Part of the fecal sample was used for the smear preparation and staining. The rest of the feces was mixed with two volumes of potassium dichromate 2.5% and was stored at 4 °C [26].

Staining

First, 816 stool samples were stained using modified trichrome (Weber) staining. The slides were fixed with methanol and placed in the trichrome stain for 240 min. After decolorization with acid-alcohol and washing with 95% ethanol, the slides were placed in absolute ethanol. Next, to identify the spores, the slides were examined by optical microscopy at 100× magnification with immersion oil. The positive samples were identified by the dorsal vacuoles in the microsporidia spores [27,28].

Extraction of DNA

The DNA was extracted using DNA stool kits (Bioneer), and the extracted DNA was stored at −20 °C. This kit consisted of spin columns that absorbed the parasite DNA and eluted the purified DNA after washing twice with special buffers [28].

Molecular detection

The extracted DNA was examined using the multiplex/nested PCR method that was used to identify the microsporidial genera of *Enterocytozoon* and *Encephalitozoon*. For PCR, we used the specific primers that were designed by Katzwinkel-Wladarsch et al. [29]. These primers were designed based on the small subunit ribosomal RNA (16S rRNA) gene that was used for the identification of different species of microsporidia. The primers were purchased from Bioneer Company and stored at −20 °C. Table 2 shows the primary and secondary primers used for the multiplex/nested PCR method. The lengths of the fragments amplified by the primers were 500 bp and 300 bp for the microsporidial genera of *Enterocytozoon* and *Encephalitozoon*, respectively. First, the samples were examined with the primary and secondary primers using the multiplex/nested PCR method. Then, the RFLP method with Mnl1 was used to differentiate the species of *Encephalitozoon* in the multiplex/nested PCR products [28].

Table 2
The primary and secondary primers used for the multiplex/nested PCR [29].

Primary primers	Secondary primers
MSP-1: TGAATGKGTCCCTGT	MSP-3: GGAATTCACACCGCCCGT C(A,G)(C,T) TAT
MSP-2A: TCACTCGCCGCTACT	MSP-4A: CCAAGCTTATGCTTAAGT (C,T)(A,C)AA(A,G)GGGT
MSP-2B: GTTCATTCGCACTACT	MSP-4B: CCAAGCTTATGCTTAAGTCCAGGGAG

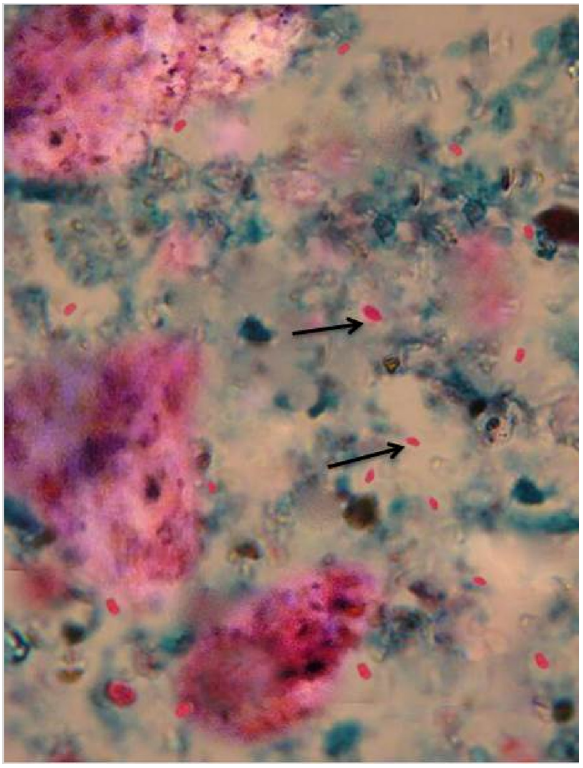

Fig. 1. The microsporidia spores in the stool sample of exotic birds that were stained by Weber staining and examined microscopically at a magnification of 100×.

Sequencing

For genotyping, the positive samples from the RFLP assay were sequenced by the Bioneer Company (Daejeon, South Korea). Afterwards, the specified sequence was compared with the sequences of the registered isolates available in the GenBank library (NCBI), and the homology between sequences was examined using BLAST software [28].

Results

Staining

Fig. 1 demonstrates the stained microsporidia spores in the stool samples of exotic birds. Of 816 samples, 138 cases were suspected to be positive for the parasite spore by the staining method, and of these 138 samples, 119 cases were verified as positive by the

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Table 3
The results of the molecular analysis of stool samples from exotic birds.

Birds	Number	Positive	*E. bieneusi*	*Encephalitozoon* spp.	*E. intestinalis*	*E. hellem*	*E. cuniculi*
Budgerigars	180	52	33	19	12	3	4
Myna	130	28	12	16	13	1	2
White-eared Bulbul	150	13	10	3	1	0	2
Canary	120	43	21	22	9	5	8
African grey parrot	41	9	8	1	1	0	0
Psittacidae	103	21	8	13	12	0	1
Fringillidae	92	15	11	4	4	0	0
Total	816	181	103	78	52	9	17

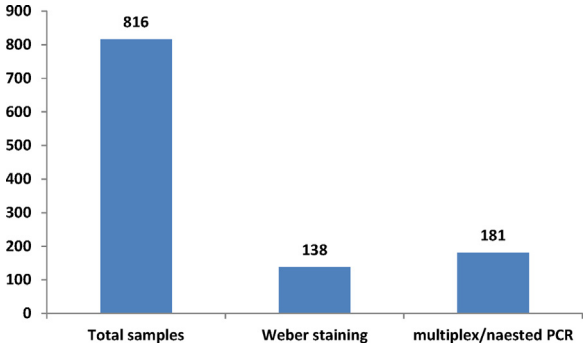

Fig. 2. The comparison of the staining and multiplex/nested PCR methods in the detection of microsporidia spores.

Fig. 3. Electrophoresis of the SSU rRNA gene PCR products on a 1.5% agarose gel, M: DNA marker 100 bp, sample 1: *Encephalitozoon* spp. positive control, sample 2: negative control, samples 3, 4: *Encephalitozoon* spp. positive samples, sample 6: *E. bieneusi* positive control, samples 7–9: *E. bieneusi* positive samples, samples 5, 10–11: negative samples.

multiplex/nested PCR method. Fig. 2 shows the comparison of the staining and multiplex/nested PCR methods for the detection of microsporidia spores.

Molecular analysis and genotyping

Fig. 3 shows the electrophoresis of the SSU rRNA gene PCR products on a 1.5% agarose gel. Fig. 4 shows the electrophoresis of the RFLP products on a 2% agarose gel. Moreover, Table 3 presents the results of molecular detection of the stool samples obtained from

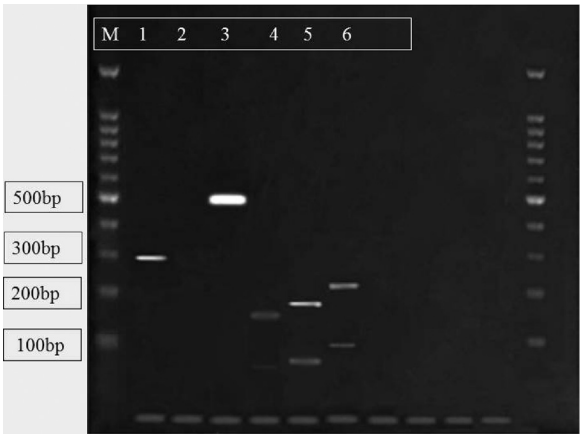

Fig. 4. Electrophoresis of the RFLP products on a 2% agarose gel.
M: 100 bp Marker, 1: *Encephalitozoon* spp. without enzyme, 2: negative control, 3: *E. bieneusi* without enzyme, 4: *E. intestinalis* (160 and 60 bp), 5: *E. hellem* (180 and 80 bp), 6: *E. cuniculi* (210 and 90 bp).

exotic birds. According to these findings, of 816 samples, 181 samples were found to be positive by multiplex/nested PCR. *E. bieneusi* and *Encephalitozoon* spp. were detected in *103 and 78 samples*, respectively. Of the *Encephalitozoon* species, there were 17 *E. cuniculi*, 52 *E. intestinalis* and 9 *E. hellem*. Of 103 *E. bieneusi* samples, 57, 39, 2 and 5 cases were detected as genotypes D, M, E and L, respectively.

Discussion

Different infectious diseases can threaten public health [30]. For example, human microsporidiosis is life-threatening to immunodeficient patients [2,7]. Hence, given the zoonotic potential of the microorganisms as well as the increasing prevalence of immunodeficiency diseases [31], the aim of this study was to evaluate molecular diagnosis of *E. bieneusi* and *Encephalitozoon* spp. in exotic birds of southwestern Iran. Since these birds have indirect and direct contacts with humans, we selected exotic birds for evaluation in this research. We used the Weber staining protocol according to Ryan et al. [32]. In this protocol, the blue aniline stain has better contrast with fungal agents and bacteria [32]. PCR methods are successful techniques that can identify microsporidia parasites with small numbers of spores. Based on different studies, the detection threshold of the microsporidia spores is 100 and 10,000–1,000,000 spores per gram of feces by PCR and light microscopy methods, respectively [33,34]. Therefore, we observed that of 816 specimens, 138 cases were positive for microsporidia spores using a staining protocol, but with the multiplex/nested-PCR method, 181 of the 816 samples were positive.

The highest prevalence of human microsporidiosis is related to *E. bieneusi*. In the current study, *E. bieneusi* also had the highest prevalence in exotic birds. The results of the research showed that 103 out of 181 fecal samples were infected with *E. bieneusi*

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(Table 3). Consistent with these results, Kemp and Kluge had reported microsporidiosis in exotic birds for the first time in 1975 [35]. In 2002, Reetz et al. detected *E. bieneusi* in nonmammalian hosts (chickens) for the first time [36]. Consistent with our findings, *E. bieneusi* had the highest prevalence in the birds in all of these studies. *E. bieneusi* has been identified in chickens, grey parrots, pigeons, cockatiels, lovebirds, finches, falcons and other birds [10,19–23]. In addition, we indicated that of 103 *E. bieneusi* samples, 57, 39, 2 and 5 cases were detected as genotypes D, M, E and L, respectively. Consistent with our results, it has been suggested that genotype D is the most common genotype in most investigations [37], which could be associated with the potential prevalence of the genotype [28].

We detected that 78 samples were infected with *Encephalitozoon* spp., including 17 *E. cuniculi*, 52 *E. intestinalis* and 9 *E. hellem*. *E. intestinalis* has also been detected in pigeons and geese [9–11]. In contrast to these studies and our study, Kašičková et al. reported that microsporidial DNA was detected in 115 fecal samples in 2009 (40.1%). There were 36 birds (12.5%) infected with *E. bieneusi*, 36 birds (12.5%) infected with *E. cuniculi* and 18 birds (6.3%) infected with *E. hellem*. None of the samples were positive for *E. intestinalis*. In addition, co-infections were identified in 25 birds: *E. bieneusi* together with *E. hellem* in 11 cases (3.8%) and with *E. cuniculi* in 14 birds (4.9%) [38]. Moreover, in 2007, Kašičková et al. showed that *E. cuniculi* had the highest prevalence in cockatiels [17]. *E. cuniculi* has also been detected in chickens and pigeons [10,18], and *E. hellem* has been found in ducks, pigeons, geese, crows, puffins, hummingbirds, swans and cranes [1,8–11] and in captive birds from the order Psittaciformes, which includes lovebirds, budgerigars, Eclectus parrots, parrots, cockatoos and lories [1,12–16].

This research is important and significant in terms of public health because the opportunistic pathogens and parasites were isolated from exotic birds in southwestern Iran. Because of the indirect and direct relationships of microsporidia with humans, these exotic birds are an important source of contamination. Furthermore, it is recommended to investigators to evaluate the various hosts as well as the role of the hosts in infecting different individuals. In conclusion, the results showed a relatively high prevalence of microsporidia in exotic birds, and according to the results of genotyping, these birds can be an important source of microsporidiosis. Thus, for the design of proper precautionary programs, it is essential that high-risk individuals, including patients with immunodeficiency diseases, receive accurate and valid information about the risk of direct and indirect contact with infected exotic birds.

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Competing interests

None declared.

Ethical approval

Not required.

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References

[1] Sak B, Kašičková D, Kváč M, Květoňová D, Ditrich O. Microsporidia in exotic birds: intermittent spore excretion of *Encephalitozoon* spp. in naturally infected budgerigars (*Melopsittacus undulatus*). Vet Parasitol 2010;168(3):196–200.
[2] Franzen C, Müller A. Molecular techniques for detection, species differentiation, and phylogenetic analysis of microsporidia. Clin Microbiol Rev 1999;12(2):243–85.
[3] Weber R, Bryan RT, Schwartz DA, Owen RL. Human microsporidial infections. Clin Microbiol Rev 1994;7(4):426–61.
[4] Didier ES, Snowden KF, Shadduck JA. Biology of microsporidian species infecting mammals. Adv Parasitol 1998;40:283–320.
[5] Omalu I, Duhlinska D, Anyanwu G, Parn V, Inyama P. Human microsporidial infections. Online J Health Allied Sci 2006;3(2).
[6] Lindsay DS, Weiss LM. Opportunistic infections: toxoplasma, sarcocystis and microsporidia. Springer Science & Business Media; 2004.
[7] Samie A, Obi C, Tzipori S, Weiss L, Guerrant R. Microsporidiosis in South Africa: PCR detection in stool samples of HIV-positive and HIV-negative individuals and school children in Vhembe district, Limpopo Province. Trans R Soc Trop Med Hyg 2007;101(6):547–54.
[8] Snowden K, Daft B, Nordhausen RW. Morphological and molecular characterization of *Encephalitozoon* hellem in hummingbirds. Avian Pathol 2001;30(3):251–5.
[9] Haro M, Izquierdo F, Henriques-Gil N, Andrés I, Alonso F, Fenoy S, et al. First detection and genotyping of human-associated microsporidia in pigeons from urban parks. Appl Environ Microbiol 2005;71(6):3153–7.
[10] Bart A, Wentink-Bonnema EM, Heddema ER, Buijs J, van Gool T. Frequent occurrence of human-associated microsporidia in fecal droppings of urban pigeons in Amsterdam, the Netherlands. Appl Environ Microbiol 2008;74(22):7056–8.
[11] Slodkowicz-Kowalska A, Graczyk TK, Tamang L, Jedrzejewski S, Nowosad A, Zduniak P, et al. Microsporidian species known to infect humans are present in aquatic birds: implications for transmission via water? Appl Environ Microbiol 2006;72(7):4540–4.
[12] Black S, Steinohrt L, Bertucci D, Rogers L, Didier E. *Encephalitozoon* hellem in budgerigars (*Melopsittacus undulatus*). Vet Pathol 1997;34(3):189–98.
[13] Gray M, Puette M, Latimer K. Microsporidiosis in a young ostrich (*Struthio camelus*). Avian Dis 1998;83:2–6.
[14] Pllparampil N, Graham D, Phalen D, Snowden K. *Encephalitozoon* hellem in two eclectus parrots (*Eclectus roratus*): identification from archival tissues. J Eukaryot Microbiol 1998;45(6):651–5.
[15] Snowden K, Logan K. Molecular identification of *Encephalitozoon* hellem in an ostrich. Avian Dis 1999;77:9–82.
[16] Suter C, Mathis A, Hoop R, Deplazes P. *Encephalitozoon* hellem infection in a yellow-streaked lory (*Chalcopsitta scintillata*) imported from Indonesia. Vet Record 1998;143(25):694–5.
[17] Kašičková D, Sak B, Kváč M, Ditrich O. Detection of *Encephalitozoon* cuniculi in a new host—cockateel (*Nymphicus hollandicus*) using molecular methods. Parasitol Res 2007;101(6):1685–8.
[18] Reetz J. Natural transmission of microsporidia (*Encephalitozoon cuniculi*) by way of the chicken egg. Tierarztliche Praxis 1994;22(2):147–50.
[19] Lobo ML, Xiao L, Cama V, Magalhães N, Antunes F, Matos O. Identification of potentially human-pathogenic *Enterocytozoon bieneusi* genotypes in various birds. Appl Environ Microbiol 2006;72(11):7380–2.
[20] Graczyk TK, Sunderland D, Rule AM, da Silva AJ, Moura IN, Tamang L, et al. Urban feral pigeons (*Columba livia*) as a source for air-and waterborne contamination with *Enterocytozoon bieneusi* spores. Appl Environ Microbiol 2007;73(13):4357–8.
[21] Haro M, Henriques-Gil N, Fenoy S, Izquierdo F, Alonso F, Del Aguila C. Detection and genotyping of *Enterocytozoon bieneusi* in pigeons. J Eukaryot Microbiol 2006;53 (s1).
[22] Li W, Tao W, Jiang Y, Diao R, Yang J, Xiao L. Genotypic distribution and phylogenetic characterization of *Enterocytozoon bieneusi* in diarrheic chickens and pigs in multiple cities, China: potential zoonotic transmission. PloS One 2014;9(9):e108279.
[23] Zhao W, Yu S, Yang Z, Zhang Y, Zhang L, Wang R, et al. Genotyping of *Enterocytozoon bieneusi* (Microsporidia) isolated from various birds in China. Infect Genet Evol 2016;40:151–4.
[24] Deplazes P, Mathis A, Weber R. Epidemiology and zoonotic aspects of microsporidia of mammals and birds. *Cryptosporidiosis* and microsporidiosis, 6. Karger Publishers; 2000. p. 236–60.
[25] Yee RW, Tio FO, Martinez JA, Held KS, Shadduck JA, Didier ES. Resolution of microsporidial epithelial keratopathy in a patient with AIDS. Ophthalmology 1991;98(2):196–201.
[26] Cama VA, Pearson J, Cabrera L, Pacheco L, Gilman R, Meyer S, et al. Transmission of *Enterocytozoon bieneusi* between a child and guinea pigs. J Clin Microbiol 2007;45(8):2708–10.
[27] Pirestani M, Sadraei J, Forouzandeh Moghadam M. Detection and genotyping of human-associated microsporidia in pigeon *Columba livia* of Tehran in 2010. Modares J Med Sci Pathobiol 2011;14(3):15–24.
[28] Tavalla M, Mardani-Kateki M, Abdizadeh R, Nashibi R, Rafie A, Khademvatan S. Molecular identification of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in immunodeficient patients in Ahvaz, Southwest of Iran. Acta Trop 2017.
[29] Katzwinkel-Wladarsch S, Lieb M, Heise W, Löscher T, Rinder H. Direct amplification and species determination of microsporidian DNA from stool specimens. Trop Med Int Health 1996;1(3):373–8.

Please cite this article in press as: Tavalla M, et al. Molecular diagnosis of potentially human pathogenic *Enterocytozoon bieneusi* and *Encephalitozoon* species in exotic birds in Southwestern Iran. J Infect Public Health (2017), http://dx.doi.org/10.1016/j.jiph.2017.07.028

[30] Safi M, Tavalla M, Mardani M, Afrisham R. Prevalence of intestinal parasitic infections among applicants for health cards attending Ahvaz East Health Center during 2012–2013. Asian Pacific J Trop Dis 2016;6(2):151–4.
[31] Mathis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. Clin Microbiol Rev 2005;18(3):423–45.
[32] Ryan NJ, Sutherland G, Coughlan K, Globan M, Doultree J, Marshall J, et al. A new trichrome-blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. J Clin Microbiol 1993;31(12):3264–9.
[33] Müller A, Bialek R, Kämper A, Fätkenheuer G, Salzberger B, Franzen C. Detection of microsporidia in travelers with diarrhea. J Clin Microbiol 2001;39(4):1630–2.
[34] Garcia LS. Laboratory identification of the microsporidia. J Clin Microbiol 2002;40(6):1892–901.
[35] Kemp R, Kluge J. *Encephalitozoon* sp. in the Blue-Masked Lovebird, *Agapornis personata* (Reichenow): first confirmed report of microsporidan infection in birds. J Protozool 1975;22(4):489–91.
[36] Reetz J, Rinder H, Thomschke A, Manke H, Schwebs M, Bruderek A. First detection of the microsporidium *Enterocytozoonbieneusi* in non-mammalian hosts (chickens). Int J Parasitol 2002;32(7):785–7.
[37] Thellier M, Breton J. *Enterocytozoon bieneusi* in human and animals, focus on laboratory identification and molecular epidemiology. Parasite 2008;15(3):349–58.
[38] Kaščková D, Sak B, Kváč M, Ditrich O. Sources of potentially infectious human microsporidia: molecular characterisation of microsporidia isolates from exotic birds in the Czech Republic, prevalence study and importance of birds in epidemiology of the human microsporidial infections. Vet Parasitol 2009;165(1):125–30.

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